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INVITED REVIEW

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The dendron and episodic neuropeptide release

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Abstract

The unexpected observation that the long processes of gonadotrophin-releasing hormone (GnRH) neurons not only conducted action potentials, but also operated to integrate afferent information at their distal-most extent gave rise to the concept of a blended dendritic-axonal process termed the "dendron". The proximal dendrites of the GnRH neuron function in a conventional manner, receiving synaptic inputs and initiating action potentials that are critical for the surge mode of GnRH secretion. The distal dendrons are regulated by both classical synapses and volume transmission and likely operate using subthreshold electrotonic propagation into the nearby axon terminals in the median eminence. Evidence indicates that neural processing at the distal dendron is responsible for the pulsatile patterning of GnRH secretion. Although the dendron remains unique to the GnRH neuron, data show that it exists in both mice and rats and may be a common feature of mammalian species in which GnRH neuron cell bodies do not migrate into the basal hypothalamus. This review outlines the discovery and function of the dendron as a unique neuronal structure optimised to generate episodic neuronal output.

KEYWORDS

axon, dendrite, fertility, fertility, GnRH, kisspeptin, luteinising hormone, pulse

1 | INTRODUCTION

Cajal established the principle that interconnections between neurons provided the functional links enabling coherent activity in the brain. As highly polarised cells, the transfer of information between neurons occurs in a stereotypical manner with electrical charge flowing from the dendrites to the soma and on to the axon. Although this pattern of information processing holds for the vast majority of neurons, exceptions exist. Invertebrate neurons do not often have defined axons and dendrites and their absence of action potential initiation segments results in the graded processing of information within their neurites.[1,2] Rarely, neurons with similar features can be found in vertebrates. For example, the axon-less amacrine neurons of the retina and olfactory bulb granule cells have neuritic arbours that receive and transmit information in local microprocessing domains.[3,4] A more common form of non-classical transmission in vertebrates is that of dendro-dendritic signalling, where dendrites release transmitters to modulate the activity of nearby dendrites. This mode of communication has now been observed in many networks but remains exemplified by the hypothalamic magnocellular neurons where dendritic neuropeptide release co-ordinates activity both within and across specific modalities.[5,6]

We reported in 2013 that murine gonadotrophin-releasing hormone (GnRH) neurons utilised a unique form of non-classical neurotransmission in which their long neural processes were not only responsible for the axon-like propagation of action potentials, but also behaved as dendrites receiving synaptic input.[7] These processes were termed "dendrons" to reflect their mixed dendritic and

axonal properties. Subsequent investigations have indicated that the distal-most aspect of the GnRH neuron dendron operates as an autonomous regulatory microdomain immediately upstream of short axons secreting GnRH into the portal circulation.[8-10] After almost a decade, the GnRH neurons remain the only example of this type of signalling in the mammalian brain. In other neurons, afferent synaptic inputs exist around the action potential initiation segment and the nerve terminal but are rarely encountered elsewhere on the axon.[11,12] Equally, it is well established that dendritic trees can support back-propagating action potentials,[13] although these dendrites do not give rise to axons with nerve terminals.

This short review aims to summarise the discovery, characteristics and role of the GnRH neuron dendron in the mouse and highlight the key ongoing challenges in understanding its utility and why it exists.

2 | DISCOVERY OF THE DENDRON

The pioneering studies of Barry and colleagues revealed that mammalian GnRH neurons exhibited a predominantly bipolar nature and were scattered throughout the basal forebrain[14] (Figure 1A). The existence of both features was explained by the discovery of the remarkable migration of GnRH neurons along olfactory axons from the nasal placode into the hypothalamus during embryogenesis.[15] Although many studies used immunohistochemistry to further characterise the GnRH neurons at the level of the light- and electron-microscope,[16] it remained that this only ever allowed examination of those parts of the GnRH neuron containing GnRH peptide. In 2003, Rebecca Campbell and Seong-Kyu Han began a series of experiments in which individual living GnRH neurons, identified in brain slices prepared from transgenic green fluorescent protein-tagged GnRH mice, were injected with biocytin allowing the entire intracellular space of the GnRH neuron to be delineated. These studies identified the presence of primary cilia and numerous spines on GnRH neurons, as well as the existence of curiously long dendrites extending from both poles of the GnRH neuron; these processes ran for up to 1000 μm before exiting the brain slice.[17] Equally remarkable was the failure to identify any clear axon-like structure emanating from the GnRH neuron.

The subsequent development of a thick horizontal brain slice by Stephanie Constantin provided a preparation in which the caudal-most GnRH neurons located within the anterior hypothalamic area (AHA) remained intact alongside an undisturbed median eminence[18] (Figure 1A). This was thought to be the ideal preparation for visualising the pathway through which axons from individual AHA GnRH neurons innervated the median eminence. To the great surprise of Michel Herde, biocytin filling of AHA GnRH neurons in these slices revealed that there were no axons but that the long dendrites, often from both poles of the GnRH neuron, ran all the way to the median eminence before breaking up into short axons that converged onto the portal vasculature. Electrophysiological studies by Karl Iremonger demonstrated typical electrical propagation by these processes and the presence of functional amino acid receptors on distal dendrites at the border of the median eminence. Taken together, the observation that GnRH neurons extended long action potential-carrying processes that also received synaptic inputs and gave rise to the concept of the dendron.[7] Not until the introduction of whole-brain clearing methodologies was it possible to demonstrate that this was not a special feature of AHA GnRH neurons;

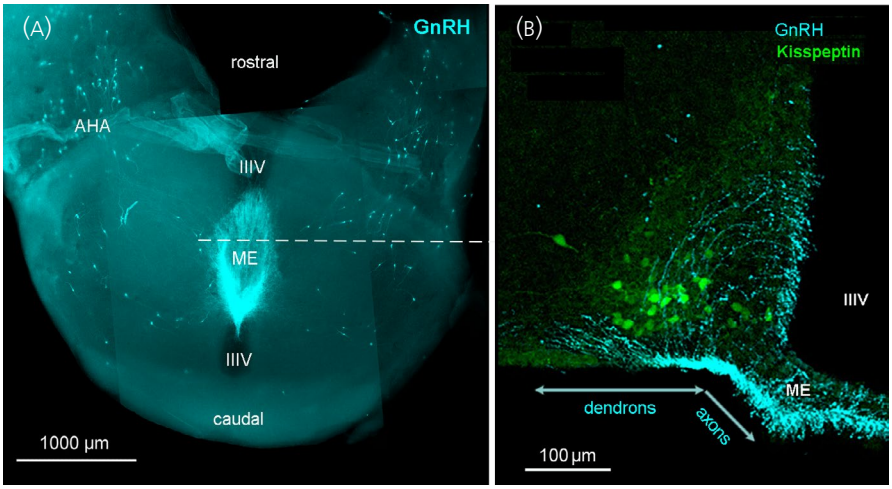

FIGURE 1 Photomicrographs showing the location of the distal dendron. A, View looking down on the base of the mouse brain in a para-horizontal brain section. The dense midline accumulation of gonadotrophin-releasing hormone (GnRH) projections around and within the median eminence (ME) is surrounded by scattered individual GnRH neurons located laterally in the anterior hypothalamic area (AHA) on both sides of the brain. The dotted line indicates the approximate coronal plane represented in (B). B, Coronal section at the level of the rostral arcuate nucleus showing the distribution of GnRH fibres (blue immunohistochemistry) in relation to the kisspeptin neuron cell bodies (genetically targeted enhanced green fluorescent protein). Note that GnRH fibres (axons) run along the wall of the third ventricle (IIIV), whereas the distal dendrons enter from the lateral aspects immediately beneath the kisspeptin neurons in the arcuate nucleus. The arrows indicate the approximate region of dendrons before they fasciculate into short axons that innervate the ME

those located in the preoptic area were also demonstrated to project exceptionally long dendrites (some for up to 5000 μm) to the base of the brain before fasciculating into short axons that entered the median eminence[19] (Figure 2).

3 | MORPHOLOGICAL CHARACTERISTICS OF THE GNRH neuron

3.1 | Proximal aspects of the GnRH neuron

Technical advances have greatly enhanced the morphological characterisation of the GnRH neuron in the mouse.[20] Before detailing the features of the dendron, it is important to provide a quick overview of our current understanding of the proximal aspects of the GnRH neuron (Figure 2). As noted above, the great majority of GnRH neurons exhibit a uni- or bipolar morphology and lie in a longitudinal orientation within the brain with each pole giving rise to a dendrite. Because it is not possible to define with certainty that every dendritic projection from a GnRH neuron becomes a dendron, they are described as "dendrites" when considering the proximal morphology of a GnRH neuron and as "dendrons" when describing the distal processes around the median eminence. One of the dendrites projecting from the GnRH cell body is often thicker than the other leading to its classification as the primary dendrite.

Cell-filling studies show that the approximately half of dendrites projecting from the dorsal pole of the cell eventually undertake a hairpin turn and head back in the same direction as the other dendrite traveling ventro-caudally towards the median eminence.[7,21] Electrophysiological assessments[22,23] and ankyrin G labelling[21] show that the GnRH neurons only have one action potential initiation segment and this is located 50-150 μm along the dendrite. Although this can be present in either dendrite, the action potential initiation segment is most commonly observed in the secondary dendrite.[21] As such, action potentials will propagate down the secondary dendrite and, after passing through the cell body, also pass down the primary dendrite. This very likely provides two outputs to the median eminence for each GnRH neuron.[7]

Synaptic inputs to proximal aspects of the GnRH neuron are clustered around either dendrite but interestingly have their highest density in the region between the action potential initiation segment and soma, rather than at the action potential initiation segment itself[17,21,24] (Figure 2). These afferents are primarily glutamatergic, although GABAergic synapses are also detected and one-third of all synapses on GnRH neurons contain kisspeptin[10,24]. One striking feature of the proximal dendrites is their tendency to bundle together such that multiple dendrites will run in the same course intertwining and separating and then intertwining again.[25] Although no evidence was found for gap junctional coupling at these sites, shared synapses with single axons synapsing on two GnRH neuron dendrites are frequently encountered.[25] This may represent a mechanism for the synchronisation of proximal GnRH neuron elements.

3.2 | Distal aspects of the GnRH neuron

Originating from the widely scattered GnRH neuron cell bodies, the dendrons pass ventro-laterally in the brain to ultimately converge on the lateral margins of the median eminence. Within approximately 100 μm of the median eminence, the dendron begins to break up into multiple small axons that pass into the median eminence that end on the portal vasculature[7,19] (Figures 1B and 2).

The dendron is the most densely innervated compartment of the GnRH neuron, having double the density of synapses compared to the proximal dendrites.[9] Electron microscopic examination of the dendron suggests that multiple different inputs exist at this level with symmetric and asymmetric synapses containing both small and large synaptic vesicles found on spines and the smooth shaft of the dendron.[19] As has been noted before in the rat,[26] no synapses were detected on GnRH neuron axons or terminals within the median eminence of the mouse.[19] The neurochemical identity of synapses on the dendron are not well established, although they likely include GABA but not glutamate, dynorphin, neurokinin B or corticotrophin-releasing factor.[7,10,27] The initial observation of glutamate responses from the dendron of AHA GnRH neurons[7] was not detected recently when dendrons from preoptic area GnRH neurons were examined.[10] Because AHA GnRH neurons have their cell bodies close to the median eminence, it is possible that the positive glutamate responses in those cells resulted from glutamatergic receptors associated with the proximal dendrite.

As the GnRH pulse generator, the arcuate nucleus kisspeptin neurons provide an extremely important input to the dendron (see below). Interestingly, kisspeptin neurons signal to the dendrons using volume transmission rather than direct synapses, as is the case for the GnRH neuron cell body and proximal dendrites.[10] Kisspeptin neurons extend intertwining projections into the dendron field that result in vesicle-containing varicosities sitting next to, but not synapsing on, multiple dendrons (Figure 2). Rather than providing tight millisecond synaptic control between two cells, this would generate a slower but more broadcast signal that would be compatible with the need to intermittently drive GnRH release for periods of minutes.[28]

It is important to note that the innervation of the dendron very likely occurs behind the blood-brain barrier (BBB) (Figure 2). The peripheral administration of Fluorogold is well established to identify neurons with projections outside the BBB. Whereas Fluorogold labels GnRH and other hypophysiotropic neurons, it is not detected in any kisspeptin neurons in rats or mice,[29,30] indicating that they are entirely behind the BBB. This anatomical arrangement in which the classical presynaptic innervation of nerve terminals appears to have been moved up behind the BBB may well protect this key signalling site from interference by blood-borne substances. For example, very

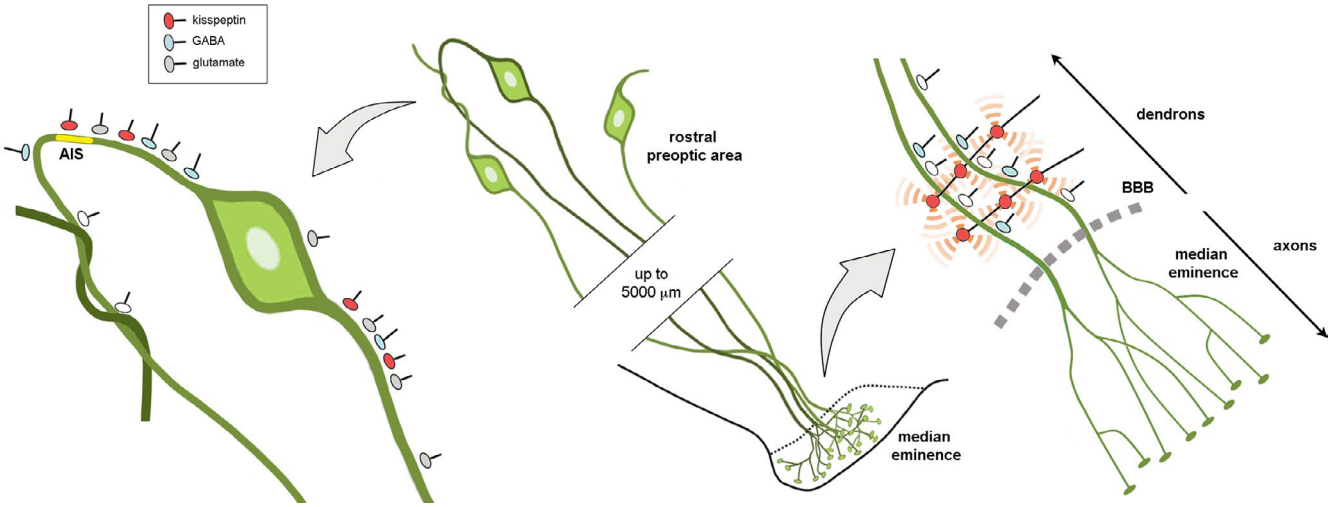

FIGURE 2 Schematic diagram depicting gonadotrophin-releasing hormone (GnRH) neuron morphology in the rodent. Left: Proximal components of the GnRH neuron including the cell body and two proximal dendrites, one or both of which may become dendrons projecting to the median eminence. The cell body, that receives relatively little synaptic innervation, has two dendrites arising from either pole with the dorsoventral dendrite often making a hairpin turn to run in the same direction as the other dendrite towards the median eminence. The axon potential initiation segment (yellow, AIS) is most often located 50-100 µm along the rostro-dorsal dendrite. Glutamatergic and GABAergic synaptic input is most dense at the proximal dendrite with shared synapses found at locations where dendrites intertwine. Right: The distal compartment of the GnRH neuron, which can be up to 5000 µm away from the cell body, is comprised of dendrons that break up into short axons that innervate the median eminence. A high density of synapses is found on the distal dendron in addition to volume transmission provided by kisspeptin fibres (red). Synapses are not found on GnRH neuron axons and synaptic inputs to the dendron very likely occur behind the blood-brain-barrier (BBB)

high levels of kisspeptin derived from the placenta are found in the circulation during late pregnancy.[31]

4 | ELECTRICAL PROPERTIES OF THE DENDRON

Electrophysiological studies show that the dendron conducts action potentials in the normal manner resulting in N-type voltage-gated calcium channel activation in GnRH axon terminals.[8] It is also clear that local puffs of kisspeptin can increase calcium levels in the dendron and axon terminals through N-type voltage-gated calcium channels.[8,10] The likelihood that kisspeptin actions at the distal dendron control GnRH secretion is further supported by the ability of kisspeptin to release GnRH from various "median eminence" preparations that include the dendrons and axon terminals.[29,32-34]

The local effects of kisspeptin on calcium concentrations in the dendron[8] and GnRH release[32,34] do not require voltage-activated sodium channels. Equally, there is no evidence for action potential initiation segments in the dendron.[7,8] This suggests that the dendron couples to GnRH release sites in the axon terminals through subthreshold membrane depolarisation. A key question is how far such electrotonic spread may occur from the distal dendrite into the axon terminal. The length constant of GnRH neuron dendrons is not known but experimental and theoretical assessments of GnRH neurondendrites show that most sub-threshold events have dissipated by 300-350 µm from the initiation site.[7,23,35] The fasciculation of the dendron into axons typically occurs within 100 µm of the median eminence so that propagated potentials would likely enter nearby axon terminals.[7,19] However some axons have a long and tortuous branching path within the median eminence[7] and it is not clear how the most distal terminals might be influenced by events at the dendron, if at all. Reconstructions of dendronic projections show that their axonal arbours are found throughout the rostro-caudal length of the median eminence.[7] However, given that axon terminals closest to the dendron will be the most directly controlled by the pulse generator, it is possible that terminals positioned more laterally in the median eminence may drive pulsatile GnRH secretion. By contrast, axon terminals throughout the median eminence would be expected to receive action potentials generated by the action potential initiation segment located in the proximal GnRH neuron compartment and contribute to the surge.

Finally, it is worth noting that sparse inputs also appear to exist on the long intermediary dendronic processes between the relatively densely innervated proximal dendrite and distal dendron.[7] Prior studies have reported that axo-axonal inputs can modulate the dynamics of propagating action potentials.[36] The same may be true for the dendron as glutamate uncaging was found to cause a small increase in action potential width as it passed through the activated region.[7] However, the impact of this on GnRH secretion, if any, remains unknown.

5 | FUNCTIONAL ROLE OF THE DENDRON

Recent studies have demonstrated that the proximal and distal compartments of the GnRH neuron are likely involved in generating the

different modes of GnRH secretion.[9] Inputs and electrical integration directed at proximal elements of the GnRH neuron are important for generating the GnRH surge, whereas independent activity at the distal dendron is responsible for driving the pulsatile pattern of GnRH secretion.

There is now substantial evidence that the arcuate nucleus kisspeptin (ARN^{KISS}) neurons target the distal dendron to generate pulses of GnRH.[37] The ARN^{KISS} neurons only project to the distal processes of the GnRH neuron[38] where they operate through volume transmission.[10] Functionally, studies conducted in vitro show that small local puffs of kisspeptin generate synchronised increases in intracellular calcium levels within multiple dendrons.[8,10,39] Interestingly, the activation of the dendron by ARN^{KISS} neurons only occurs through kisspeptin-KISS1R signalling, despite the presence of other co-expressed neuropeptides and transmitters in ARN^{KISS} neuron nerve terminals.[10] In vivo, the selective optogenetic activation of the ARN^{KISS} neurons for 1 min at 5-10 Hz is very effective at generating increases in luteinising hormone (LH) secretion that are almost identical to endogenous LH pulses.[40-42] Taken together, these data demonstrate that the key role for the distal dendron is that of responding to the ARN^{KISS} neuron pulse generator to create pulsatile GnRH secretion. This is compatible with the ability of in vitro medio-basal hypothalamic tissue, containing ARN^{KISS} neurons and dendrons but no GnRH neuron cell bodies, to exhibit pulsatile GnRH secretion.[43,44]

Given the high density of synaptic inputs at the distal dendron,[19] it appears to operate as a point of neural integration beyond the kisspeptin regulation of pulsatile GnRH secretion. However, the neurochemical identity and origin of these synapses is unknown and this remains a significant challenge given current technical limitations. Many in vitro studies in the past reported effects of different transmitters and neuropeptides on GnRH release from median eminence explants,[45] which would certainly have contained GnRH neuron dendrons. Thus, it is possible that a variety of different inputs converge on the dendron to modulate kisspeptin-driven pulses. Apart from kisspeptin, the only transmitter identified to date to have a substantial effect on the dendron is GABA, with $GABA_B$ receptor activation inducing a profound suppression of intracellular calcium levels within all GnRH neuron dendrons (Liu X & Herbison AE, unpublished data). Thus, the distal dendron represents an autonomous regulatory component of the GnRH neuron where kisspeptin drives pulsatile GnRH release and where other inputs may modulate the generation of pulses.

The above discussion provides a case for the distal dendron being a key signalling domain of the GnRH neuron in mice. However, several important questions and challenges remain, some of which are addressed below.

5.1 | Where is the GnRH neuron axon?

Much of the impetus for discovering the dendron came from the search for the GnRH neuron axon. Several electron microscopic investigations have reported the presence of GnRH-immunoreactive synapses on GnRH and other neurons.[46,47] and there is evidence for widespread expression of the GnRH receptor in the brain.[48,49] This suggests that GnRH axons should exist in the brain and, indeed, axon-like GnRH projections are observed with immunohistochemistry. On re-examining this issue, and taking a more lenient morphological definition of an axon, a subpopulation of neurobiotin-filled GnRH neurons was identified in mice that extended axon-like structures arising principally from the proximal dendrite.[21] Approximately 25% of preoptic area GnRH neurons were found to have a thin, non-spiny projection with beaded processes that might reasonably be considered an axon. This increased to around 50% when examining those GnRH neurons with cell bodies located within and adjacent to the organum vasculosum of the lamina terminalis (OVLT). It is notable that axons originating from GnRH neuron dendrites and dendrons are most plentiful immediately adjacent to highly vascularised regions such as the median eminence and OVLT. Studies conducted in vitro have indicated that soluble factors from endothelial cells are strongly chemoattractant for GnRH neuron axons.[50]

Although it appears certain that GnRH neuron axons are elaborated by a subpopulation of GnRH neurons, their roles are uncertain. As only approximately two-thirds of GnRH neurons are hypophysiotropic,[51,52] it is also unclear which GnRH neurons elaborate axons. Nevertheless, one likely possibility is that axons projecting to other brain regions enable the occurrence of the GnRH surge to be signalled to relevant networks; for example, those involved in female reproductive behaviour.[53] By contrast, the functions of the very dense GnRH neuron axon terminal projection within the OVLT remain enigmatic.[54] Another axonal pathway with a close association to the ventricular system is the medial projection that runs alongside the wall of the third ventricle.[19,55] Upon reaching the mediobasal hypothalamus, these fibres appear to cascade through the ARN (Figure 1B) and potentially, pass down into the median eminence.[55] Again, the role of this medial projection pathway is unknown and may contribute to the synaptic control of ARN networks,[56] release of GnRH into the cerebrospinal fluid[54] and/or provide a conventional axonal GnRH input to the median eminence.

5.2 | Does the dendron exist in species other than the mouse?

It was only possible to identify and characterise the dendron in mice using genetic manipulations that enabled pre-identification and selective calcium imaging in the GnRH neuronal phenotype. The recent generation of a GnRH-Cre rat made it possible to identify the existence of the dendron in this species with many dendron-like processes that respond to kisspeptin also found in the ventrolateral arcuate nucleus adjacent to the median eminence.[39] Functionally, this explains data obtained in vivo showing that kisspeptin injected into the arcuate nucleus stimulates LH secretion in rats.[57] Thus, the dendron appears to exist in both mice and rats.

A key unresolved question is whether the dendron occurs in species such as the sheep and primates where many GnRH neuron

cell bodies migrate down into the basal hypothalamus.[16] It could be argued, for example, that the arcuate kisspeptin neuron pulse generator need only innervate the nearby GnRH neuron cell bodies and dendrites in the basal hypothalamus to drive pulsatile GnRH release. Although divergent kisspeptin volume transmission has many attractions for synchronising the clustering dendrons, direct synapses to GnRH neuron dendrites could achieve the same purpose.

Evidence for the dendron in species outside the rat and mouse remains circumstantial at best. Goats are reported to have no arcuate kisspeptin inputs to any GnRH neuron cell bodies with all communication thought to occur through non-synaptic transmission around axons in the median eminence.[58] However, whether the dendron exist in goats is unknown. By contrast, studies in the sheep have found that arcuate kisspeptin neurons innervate GnRH neuron cell bodies throughout the basal forebrain including those in the basal hypothalamus.[59] Although complicated by the likely dual role of ovine arcuate kisspeptin neurons in pulse and surge generation, this suggests that conventional axo-somatic kisspeptin transmission may be involved in pulse generation in the sheep. Nevertheless, microinjection of a kisspeptin receptor antagonist into the arcuate nucleus of the sheep slows the occurrence of the next LH pulse[60] leaving open the possibility that dendrons in the ventrolateral arcuate are present. As in the rat and mouse, ovine median eminence explants respond to kisspeptin[33] demonstrating that GnRH neuron distal processes, whether axons and/or dendrons, are sensitive to kisspeptin. Furthermore, one electron microscopic study searching for synapses on GnRH neuron axons in the median eminence of sheep described synapses on GnRH neuron projections, thought likely to be dendrites, beneath the arcuate nucleus.[61]

Less information is available for the primate although overlap between kisspeptin and GnRH projections occurs at the boundaries of the median eminence.[62] In both the monkey and human, kisspeptin and GnRH fibres can be traced running together from the median eminence into the neurohypophysis[63,64] where their function remains unknown. As with sheep, arcuate kisspeptin neurons project to GnRH neuron cell bodies and dendrites in the basal hypothalamus of humans.[65] Functionally, episodic fluctuations in kisspeptin, which often correlate with pulsatile GnRH secretion, are observed in the monkey median eminence region and kisspeptin, alongside other transmitters, administered into the region of the median eminence modulates pulsatile LH secretion.[66,67] Although these observations are compatible with the presence of a dendron in primates, they could also be explained by kisspeptin innervation of the nearby basal hypothalamic GnRH neuron cell bodies and dendrites. Clearly, further studies are required to examine whether the dendron exists in non-rodent species.

5.3 | Is there any role for neural regulation of GnRH secretion at the axon terminal in the median eminence?

The assessment of the effects of different compounds on GnRH release from "median eminence" and basal hypothalamic explants was a popular in vitro experimental platform in the 1980 and 1990s.[45] Without knowledge of the existence of the dendron, observations from these studies were quite reasonably interpreted as resulting from transmitter regulation of the GnRH neuron axon terminals. However, in all cases, these experiments would have included both the distal dendrons and axon terminals and it is essentially impossible to differentiate between actions at these two sites. Electron microscope studies in mice, rats, sheep, goats and primates have all struggled to identify any synapses on GnRH neuron axon terminals in the median eminence,[19,29,58,61,68,69] although this has not excluded the possibility of regulation by volume transmission. Thus, it remains unclear whether afferent inputs regulate GnRH secretion at the axon terminal in addition to the distal dendron. The axon terminals are nonetheless important sites of regulation as documented by the glial-neuronal plasticity that occurs at this compartment.[70]

In rodents, glutamate is one possible transmitter that is only active at the terminals. There is now little evidence for actions of glutamate at the distal dendron,[10] while NMDA and kainate receptors have been identified in GnRH axon terminals in rats.[26] One approach able to differentiate between the two sites showed that kisspeptin puffs were equally effective at activating distal dendrons and axon terminals.[8] This indicates that both GnRH neuron distal dendrons and axon terminals express KISS1R although the physiological roles for kisspeptin modulation at the later are unknown. As an aside, the ability of peripheral kisspeptin to increase LH secretion most likely results from the activation of KISS1R located on these axon terminals, as well as on GnRH neuron dendrites extending outside the BBB in the organum vasculosum of the lamina terminalis.[71]

The discovery of the dendron now calls for a re-assessment of where neurotransmitters may be acting within the median eminence to regulate GnRH secretion. Unfortunately, the very close proximity of the dendron to the median eminence (approximately 100 μm) and possible confusion with dendrites of basal hypothalamic-located GnRH neurons (in relevant species) means that only techniques with sufficient spatial resolution can be used. Certainly, the "median eminence" explants of old containing both GnRH neuron dendrons and axons need to be re-evaluated.

5.4 | Is there significant plasticity at the dendron?

Kisspeptin-KISS1R signalling at the dendron appears to be relatively stable exhibiting little or no plasticity. The response of the dendron to kisspeptin does not alter across the oestrous cycle[8] or between the sexes.[10] However, very substantial differences exist in the efficacy of optogenetic ARN^{KISS} neuron activation to stimulate LH secretion; males and ovariectomised females exhibit 4-fold higher increases in LH compared with intact females.[41] Although changes in pituitary sensitivity account for part of these differences, it is possible that some of this plasticity derives from differences in the amount of releasable kisspeptin in the nerve terminals.[72] Equally, although changes in dendron sensitivity to kisspeptin do not change across the oestrous cycle, the dramatic slowing of LH pulses at

oestrus arises from the slowing of the pulse generator itself.[73] Thus, kisspeptin signalling at the dendron appears to be quite stable with dynamic changes in LH pulse generation occurring upstream from alterations in pulse generator ferquency and the amounts of releasable kisspeptin as well as downstream from changes in pituitary sensitivity to GnRH. The potential plasticity of other inputs to the dendron remain to be established.

5.5 | Why the dendron and is it unique to the GnRH neuron?

Perhaps the most perplexing question is why the dendron exists at all. One possibility is that it solves some of the problems associated with the very unusual migration of GnRH neurons into the brain. The GnRH neurons arrive at the hypothalamus relatively late in embryonic development and end up with their cell bodies being scattered throughout the basal forebrain. It may be a considerable challenge for a synchronising population such as the arcuate kisspeptin neurons to detect and then innervate a significant number of highly scattered and remote GnRH neuron cell bodies. The projection of kisspeptin fibres just a short distance to the base of the arcuate nucleus (Figure 1B) where they can intermingle with the now clustered GnRH neuron dendrons may represent a very convenient solution for synchronising GnRH release. Reciprocal interactions between kisspeptin and factors secreted by developing GnRH neuron projections likely facilitate this anatomical arrangement.[74] The presence of synaptic inputs on the distal dendrons inside the BBB may also be important in ensuring that this key regulatory zone is not exposed to blood-borne substances. Taken together, these various developmental and functional constraints can be met by a dendron-like structure.

It is worth contrasting the dendron with the other pattern generator operating on the GnRH neurons.[75] In this case, the surge generator located in the rostral periventricular region of the third ventricle (RP3V) lies immediately medial to and nearby the final position of the scattered GnRH neuron cell bodies within the rostral preoptic area. This anatomical arrangement may favour conventional afferent control of the GnRH neuron and, indeed, there is evidence for direct, conventional synaptic innervation of a subpopulation of GnRH neuron soma and proximal dendrites by RP3V kisspeptin neurons.[10,38,76]

The dendron arrangement with long dendritic processes carrying action potentials combined with an autonomous regulatory zone just prior to their fasciculation into short secretory axons remains unique to the GnRH neurons. Perhaps the closest examples are those of the axon-less amacrine and granule cells in which localised integration of afferent input and signal propagation occurs within their dendrites.[3,4] Whether other neuroendocrine output neurons may utilise a dendron-like structure is unknown. As was the case with the GnRH neurons, it is simply presumed that they all project axons to the median eminence. The only cell type that can be excluded at present is the tuberoinfundibular dopamine neurons that have been shown by cell filling to project typical axons to the median eminence.[7] The detailed anatomy of the other hypophysiotropic neuroendocrine phenotypes remains to be established. However, if the dendron is indeed a result of the unique embryonic development of the GnRH neuron, it may remain as an interesting and distinctive feature restricted to this unusual neuronal phenotype.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article because no new data were created or analysed in this study.

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